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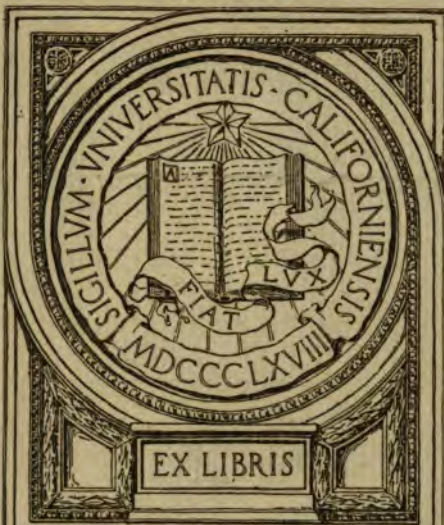


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The Occurrence of Aluminium, and its Absorp- tion from Food, in Dogs

DISSERTATION

Submitted in Partial Fulfillment of the Re-
quirements for the Degree of Doctor of
Philosophy in the Faculty of Pure Science
of Columbia University

By
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TO THE
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I.

Introduction

This paper describes an investigation of the general subject of the occurrence of aluminium in the animal body, both under normal conditions, and on diets containing aluminium.

The problem is of considerable theoretical interest. The occurrence of aluminium in the bodies of normal animals has never been specially investigated, but large amounts are certainly not present, or they would have been easily recognized ere this. This condition is in marked contrast to the abundance of aluminium in the plant and mineral worlds. That aluminium, like silicon, exists in the animal body in small amounts, could even be assumed on the ground of accidental contamination, unless some specific eliminative selection takes place, which would point biologically to an incompatibility between the metal and the organism. Absorption, and the retention of aluminium, if either or both occur, bear directly upon the possibility of substituting one element for another in the body. Information concerning such replacements consequently means an increase in our knowledge of the functions of the substances involved.

From the practical standpoint, the wide use of alum baking powders, especially in the South, makes it desirable that the effect of this aluminium should be determined.

The problem was first attacked by Papillon¹ in 1870, who, after feeding aluminium phosphate to a young rat, found that the bones contained 6.95% of alumina. He concludes that his "researches show it possible to substitute a certain quantity of strontium, of magnesium, or of aluminium for the calcium normally occurring in the bones.

H. Weiske² was unable to confirm these results with aluminium, although he verified them with regard to the other elements used. He recognizes the fact that such substitutions may not occur to a marked extent in an animal kept on a normal diet; but that by

¹Papillon: Comp. rend. de l'Acad. de sciences (Paris), lxxi, p. 372, (1870).

²H. Weiske: Zelt. fur Biologie, viii, 239, (1872), ibid. xxxi, 421, (1895)

depriving the animal of a sufficient amount of the element to be replaced, (in this case calcium) substitution may be effected.

J. Konig³ first practically applied this principle of Weiske's, using food deficient in calcium. He was, however, unable to find any large amounts of aluminium, but states that he made no attempt to look for small amounts, and concedes the possibility of their being present.

It was in this unsatisfactory condition that the problem was first taken up at this laboratory.

In 1906 House and Gies⁴ showed that a concentration of aluminium greater than 1-65,536 moles per liter markedly inhibited the growth of seedlings. It was later found by Steel⁵, working with Gies, that in dogs, aluminium passed from the alimentary tract into the blood, when they were given food mixed with alum, and that when aluminium chloride was injected intravenously this process was reversed, aluminium passing into the intestines from the blood.

Kahn⁶, also working in this laboratory, showed that aluminium is absorbed by dogs from food prepared with alum baking powder.

On the other hand, the Referee Board of Consulting Scientific Experts of the United States Department of Agriculture⁷ were unable to find aluminium in the blood of four men who "took one gram of aluminium a day for several days." The form in which the aluminium was taken, however, is not mentioned.

More recently, Leary and Sheib⁸, using dogs and white rats, which were fed with aluminium hydroxide, identified aluminium in the livers of all the animals used.

In our experiments we have used dogs, both because of their convenience, and since, of the animals available, their metabolism is nearest to that of human beings.

³J. Konig: Zeit. fur Biologie., x, 69, (1874).

⁴House and Gies: Amer. Jour. Physiol. xv, (1906). Proc. p. xix.

⁵Steel: Amer. Jour. Physiol. xxviii, 94, (1911).

⁶Kahn: Biochem. Bull. 1, 235, (1911).

⁷Bull. 103, U. S. Dept. of Agriculture.

⁸Leary and Sheib: Jour. Amer. Chem. Soc. xxxix, 1066, (1917).

II.

Method

For description of the analytical methods employed, see Balls: Biochemical Bull. V. pp. 195-202. (1916)

III.

Is the Aluminium of Aluminized Bread Soluble in the Digestive Juices?

As a preliminary to animal investigation, it was thought desirable to find out if the aluminium of aluminized bread was soluble in artificial mixtures, made to represent, as nearly as possible, those juices with which it would come in contact during the process of digestion.

To this end a sample of bread dough was made¹ and divided into three parts. The first of these was baked very thoroughly, the second could be called without exaggeration "well baked," while the third was poorly baked, the interior being quite doughy, and closely resembling ordinary bread. The difference in degree of baking is shown by the appended summary of data for the moisture content, determined on one-gram samples by drying to constant weight in a vacuum oven at 75. °C.

Table 1.

Moisture content of bread samples. (% H₂O)

Very well baked.	Well baked.	Poorly baked.
5.84%	7.02%	16.14%
5.84%	7.16%	16.04%

These different grades of bread were mixed with the extraction media used, in the proportion of 5 grams per liter. The mixtures were kept in an incubator at body temperature for twenty-four hours, and shaken ten times during that period. The solid material remaining was then filtered out, and the aluminium in the solution determined according to the following procedure:

¹Approximately of this composition:

Flour, 550. grams.
Sodium Chloride, 8. grams.
Sugar, 5. grams.
Baking Powder, 16. grams
Water, 400. grams.

The baking powder used was "Parrot and Monkey Brand," an alum baking powder purchased in the open market. It contained, according to the statement on the label, 81% anhydrous sodium alum. This was found to be correct.

The solution, first partially evaporated in a porcelain dish, was taken to dryness in a platinum one. The material was then gently ignited in an electric muffle until completely ashed. This ash was fused with approximately six times its weight of a mixture of potassium and sodium carbonates, and the melt dissolved in hydrochloric acid solution. Repeated evaporations with HCl were made to dehydrate the silica. The residue was finally moistened with cold concentrated acid; after standing some minutes diluted with water, warmed, and the silica, always small in amount, filtered off. The aluminium present in the acid solution was determined by the procedure of Schmidt and Hoagland^a. This method depends in principle upon the precipitation of aluminium as phosphate from a solution containing phosphate, whose acidity has been reduced by adding, first, ammonium thiosulphate, then ammonium acetate and acetic acid. Reprecipitation under similar conditions is necessary, otherwise the precipitate will be contaminated by iron and calcium. The precipitate is first heated gently to drive off the accompanying sulphur, then ignited and weighed as AlPO_4 .

This method gives low results, but its advantages lie in affording a direct determination of aluminium in the presence of the iron and phosphate always found in biological material. The method, as applied to small amounts of aluminium occurring in biological substances, was found by Howe^b and others in this laboratory to give satisfactory results.

Mellor^c claims that water decomposes aluminium phosphate slightly into a soluble acid phosphate and an insoluble basic phosphate, thus giving low results. This is probably merely an empirical way of describing a hydrolytic process. By washing the precipitates with dilute ammonium phosphate solution instead of with hot water as directed by Schmidt and Hoagland, we found, as one would naturally expect from the common ion effect, that less material was lost. A final washing with ammonium nitrate solution, however, is advisable, as the presence of much excess of ammonium phosphate in the precipitate requires very high and prolonged ignition to remove.

The bread samples were extracted in the following four ways:

I. With distilled water.

II. With a 0.2% solution of hydrochloric acid. (Represent-

^aSchmidt and Hoagland: Jour. Biol. Chem. **x1**, 387, (1912). See also: Biochem. Bull. **v**, (1916).

^bHowe: Biochem. Bull. **v**, 158, (1916).

^cMellor: Treatise in Inorganic Analysis. p. 608, (1913).

ing the acid concentration found in the gastric juice.)

III. With a 0.2% solution of hydrochloric acid, to which was added 0.1% of a commercial preparation of pepsin. This mixture is the so-called "artificial gastric juice," and its action will represent fairly completely the digestive processes in the stomach.

IV. After allowing the bread to digest for 24 hours in artificial gastric juice, sufficient sodium carbonate was added to furnish an excess of 5 grams per liter⁵, 0.2% of a commercial preparation of trypsin was then added, and the mixture allowed to incubate for another 24 hours.

Each determination was made in duplicate with the results shown in the following table.

Table II.

Data showing the amounts of aluminium extracted from "aluminized bread" by different solvents, and the relation of these amounts to the total quantity of aluminium in the bread. Under Column I. is given a description of the sample of bread used, and under Column II. is a description of the extraction medium. The weight AlPO_4 in milligrams, representing the aluminium extracted from 2.5 grams of bread by 500. c.c. of the extraction medium is given under Column III., while Column IV. gives the mean of the duplicate determinations, expressed in terms of the percentage of the total aluminium present, extracted by the extraction medium.

$$\text{dium. } \left(= \frac{\text{Al. extracted}}{\text{Total Al}} \times 100. \right)$$

I.	II.	III.	IV.
Very well baked bread.	Distilled water	0.2 mg.	
		0.2	2.0%
	0.2% HCl	9.9	
		10.5	86.0%
	0.2% HCl + 0.1% pepsin	11.6	
		10.8	93.0%
	0.2% HCl + 0.1% pepsin followed by 0.5% Na_2CO_3 + 0.2% trypsin	1.1	
	0.2% HCl. Al determined in dialysate, instead of in filtrate.	1.4 9.1 9.8	11.0% 79.0%

⁵Representing the usual alkalinity of the duodenal juices.

	0.2% HCl + 0.1% pepsin	10.2	
	Al determined in dialysate.	9.6	83.0%
	Total aluminium in bread sample.	12.0	
	(2.5 grams)	12.0	(100.0%)
Well baked bread	Distilled water.	0.9 mg.	
		0.8	5.0%
	0.2% HCl	10.9	
		10.3	93.0%
	0.2% HCl + 0.1% pepsin	11.4	
		11.5	100.0%
	0.2% HCl + 0.1% pepsin followed by 0.5% Na ₂ CO ₃ + 0.2% trypsin.	0.8	
		0.7	7.0%
	Total aluminium in bread sample.	11.5	
	(2.5 grams)	11.3	(100.0%)
Poorly baked bread	Distilled water	1.0 mg	
		0.8	8.0%
	0.2% HCl.	9.4	
		8.8	85.0%
	0.2% HCl + 0.1% pepsin	10.0	
		10.5	96.0%
	0.2% HCl + 0.1% pepsin followed by 0.5% Na ₂ CO ₃ + 0.2% trypsin.	1.0	
		1.2	10.0%
	Total aluminium in bread sample.	10.5	
	(2.5 grams)	10.9	(100.0%)
Poorly baked bread + an equal weight of lean meat.	0.2% HCl + 0.1% pepsin	10.2 mg.	
		10.4	96.0%
	0.2% HCl + 0.1% pepsin	7.4	
	Al determined in dialysate.	7.4	69.0%
	0.2% HCl + 0.1% pepsin followed by 0.5% Na ₂ CO ₃ + 0.2% trypsin.	1.0	
		0.7	8.0%

Inspection of this table will show that but little aluminium of the bread is extracted by water, the solubility increasing, however, with decreasing thoroughness of the baking. In the alkali-trypsin mixture the amount dissolved is also very small, although larger than with water. The same relationship also exists between

solubility and degree of baking. With hydrochloric acid the extraction is about two-thirds of the total, and the artificial gastric juice dissolves out practically all the aluminium present, the amount dissolved out being, in both of these cases, independent of the degree of baking which the material had undergone.

With the acidic extraction media the aluminium is dissolved in great part, but not entirely, to form a true solution. This can be seen by comparing, in Table II, the amount of aluminium that passes through the ordinary filter paper used in filtering off the insoluble bread residue, with that dialysed from a similar mixture through a collodion membrane. This dialysis increases the manipulative error and was therefore not applied to the trypsin-alkali and the water extracts, where the quantities of available aluminium were much smaller. By adding to the bread, in repetitions of the experiment described, an equal weight of lean meat, finely divided, the presence of a quantity of meta-protein, proteoses, and peptones from digested meat was found not to affect the results obtained from the bread alone, except to increase the amount of aluminium not in true solution and therefore not dialysable, but still capable of passing through a filter paper. This difference is more strikingly indicated in subsequent results on aluminium solutions containing large quantities of peptone, where fully half of aluminium in "solution" could not be dialysed out.

In those cases where the solubility is small, the percentages dissolved are, of course, useful only for comparison. The weights of material dissolved probably represent the values, under the conditions of the experiment, for a saturated solution of the aluminium compound in question (whatever that may be). The total amount dissolved is therefore merely a function of the dilution as long as any solid remains.

A series of experiments similar to those above, were also made on a solution of aluminium phosphate, since the phosphate is the form in which previously ionic aluminium would be found after entering the intestine, and it was accordingly decided to use this substance in feeding some of our animals.

One gram of freshly precipitated, thoroughly washed, moist aluminium phosphate^a, was dissolved in a liter of 0.2% HCl. On the addition of 20. grams of Witte peptone no precipitation occurred, indicating that proteoses and peptones would not interfere with the solubility of aluminium in the stomach. The aluminium

^aThe material contained 84.03% H₂O. An amount equal to one gram of the dried material was employed.

was only partially in true solution, as shown by the fact that only about half of it was dialysable. The aluminium was again precipitated when an excess of alkali was added equal to the percentage of alkali found in the intestines. The addition of the alkali as above, but in the presence of bile salts (0.2% sodium taurocholate was used) was productive of no difference in results. The following table summarizes these findings.

Table III.

In each case the solution used consisted of one gram of AlPO_4 dissolved in a liter of dilute HCl , to which the peptone was added as described above. Under Column I is a description of the subsequent treatment of this solution, of which 50. c.c. were used in the determinations under 1., and 100. c.c. portions in the determinations under 2. and 3. Column II. expresses the weight in milligrams of AlPO_4 in aliquot parts of the solutions after the specified treatment. Under Column III. is given the percentage of the

total AlPO_4 , which remains in solution. ($= \frac{\text{Al. dissolved}}{\text{Total Al.}} \times 100.$)		
I.	II.	III.
1. Dialysed through collodion.	24.3 mg.	
Dialysate used.	24.3	49.0%
2. 0.5% Na_2CO_3 added in excess of amount necessary to neutralize acid. Filtrate used	0.6	
	0.6	0.6%
3. 0.2% Na taurocholate + excess of 0.5% Na_2CO_3 added.	0.9	
Filtrate used.	0.7	0.8%

We conclude, then, that aluminium is undoubtedly rendered soluble and absorbable, both from aluminized bread and from aluminium phosphate by the gastric juice, and that such soluble aluminium is not entirely rendered insoluble by the alkaline intestinal fluids. The locality of this absorptive process also becomes evident. Some absorption is to be expected from the stomach during the digestion of aluminized food, for the gastric juice will contain at that time, a high concentration of aluminium. Since the stomach is not particularly well adapted to absorptive processes however, most of the soluble aluminium will find its way into the intestine, where a great part will again be rendered insoluble. This process, however, cannot be instantaneous. The upper duo-

denum is known to be frequently acid, and here absorption may be rapid, while in the lower portions of the intestines the amount of metal taken up by the body is probably negligible.

IV.

Does Aluminium Occur in the Tissues of Normal Dogs?

No observations of the occurrence of aluminium in normal animal tissues have been recorded. It is probable that while any considerable quantities, if present, would have been discovered, at the same time traces might have been as easily overlooked, especially when the attention of investigators was not particularly directed to this end.

Before determining whether aluminium is absorbed by the dog from food containing it in potentially soluble form, it was necessary, therefore, to ascertain whether aluminium exists normally in dog tissue, and if so, to what extent.

Accordingly, dogs were selected which had been fed for a period of about two months on a normal diet. This consisted of meat, cracker meal, lard, and bone ash¹, samples of which constituents, when extracted with artificial gastric juice, according to the methods described in the previous sections, failed to give a filtrate containing aluminium.

From 200. to 500. grams of blood were removed from each dog through a femoral artery, under local anaesthesia, and the dogs afterwards maintained on the same diet. Three weeks later two of the dogs were bled to death, the operation being conducted as before. After exsanguination was nearly complete, physiological salt solution was admitted to a femoral vein, and the perfusion continued until practically all of the blood had been washed out of the body. The washings were discarded. This perfusion is necessary, for were it not done, and the blood later found to contain aluminium, then all the other tissues would contain aluminium in proportion to their blood contents (which is not accurately determinable) in addition to any stored by the tissue itself.

Various parts of these two animals were then selected and prepared for analysis. The parts selected were those in which aluminium might be expected, for physiological reasons, to occur. They are also intended for direct comparison with those dealt with

¹Hashed lean beef 15. grams per kilo of body weight.
Cracker meal 2. grams per kilo of body weight.
Lard 3. grams per kilo of body weight.
Bone ash 1. gram per kilo of body weight.

in section V, where these selections will be found to have greater biological significance. Here we are merely concerned with the presence or absence of aluminium.

The tissues were freed from organic matter in one of the following ways: 1. Decomposition of the material according to Neumann, with nitric and sulphuric acids; removal of any nitric acid by boiling with water, and evaporation of the excess of sulphuric acid from a silica or platinum dish. 2. Ignition in platinum, and subsequent fusion of the ash with sodium and potassium carbonates.

The residues were then treated precisely as those obtained from the bread extracts, previously described, and the aluminium determined as phosphate. In the case of bone, however, the fusion with alkali carbonate is not necessary. Here also the procedure of Schmidt and Hoagland must be modified. The large quantity of calcium phosphate present makes necessary a greater dilution of the solution for the first precipitation. Comparatively small samples of the material must also be used, even at the sacrifice of accuracy. Working with 2.5 grams of bone ash, representing about four times that weight of fresh bone, the solution in which aluminium phosphate is first precipitated should measure about 800. c.c., and the quantities of reagents be regulated accordingly. Otherwise calcium phosphate will contaminate any precipitate of aluminium phosphate. The following results were obtained.

TABLE IV.

Data showing the aluminium content of normal dog tissues

Column I. designates the animal used; Column II. the part of the animal used; Column III. the weight of the part in grams; Column IV. the method of decomposition of the tissues; Column V. the weight of AlPO_4 found¹ in milligrams; Column VI. the aluminium calculated to Al_2O_3 , and Column VII. the aluminium calculated to milligrams of Al_2O_3 per 100. grams of the original material.

¹Six blank determinations, using the same reagents and glassware were run parallel to those recorded here. They gave the following results in milligrams of AlPO_4 :

Neumann Method.	Ignition Method
0.2 mg.	0.3 mg.
0.3	0.3
0.2	0.3

The proper corrections have accordingly been subtracted from each weight recorded in this column.

I.	II.	III.	IV.	V.	VI.	VII.
C.	Blood	588.	g. Neumann	0.1 mg.	0.04 mg.	0.01 mg.
D.	"	513.	"	1.1	0.46	0.09
G.	"	330.	"	0.0		
H.	"	340.	"	0.0		
E.	" 1st bleed'g	235.	"	0.1		
"	" 2d bleed'g	445.	"	0.0		
"	Spleen	13.	"	0.1		
"	Kidneys (2)	38.			determination lost	
"	Bile + Gall					
	Bladder	15.	"	0.0		
"	Muscle (Leg)	60.	"	0.0		
"	Claws	3.5	Ignition	0.1		
"	Bone (Femur)	10.	"	0.0		
"	Liver				determination lost	
F.	Blood					
	1st bleeding	230.	Neumann	0.1		
	2d bleeding	320.	"	0.1		
"	Spleen	10.	"	0.0		
"	Kidneys (2)	35.	"	0.1		
"	Bile+ Gall					
	Bladder	6.	"	0.0		
"	Muscle (Leg)	60.	"	0.0		
"	Claws	3.	Ignition	0.1		
"	Bone (Femur)	10.	"	0.1		
"	Ferratin, from liver	0.130	"	0.0		
"	Remainder of liver	200.	Neumann	0.0		

In but one instance was the amount of aluminium phosphate obtained large enough to exceed a reasonable error in making the weighings. In this case the blood undoubtedly contained aluminium, the presence of which was confirmed by the qualitative tests described later. Unfortunately, the animal died after the first bleeding and the body was inadvertently discarded before aluminium was known to be present in the blood. It was therefore impossible to continue work on the other tissues of this particular dog.

We regard a contamination of the material during the course of analysis, and accidental errors in the manipulation, as very unlikely, but, of course, as possibilities. The suggestion is also to be considered that the animal, whose previous history is unknown, may have eaten aluminized food prior to the beginning of our ex-

periment. The fact that this single result is directly at variance with all the others, leads us to regard it as exceptional, to say the least.

It is reasonable to suppose that most of the elements could be found in living matter if our means for their detection were delicate enough. From these results, however, we may conclude that aluminium is present in the normal dog in amounts that are too small usually for detection.

V.

Is Aluminium Absorbed from Food Containing it in Potentially Soluble Form?

Our work has shown aluminium to be absorbable from bread, containing it supposedly as the hydroxide. Some light on the absorption of aluminium from the digestive tract was obtained in section III. We wish, now, after referring to the findings of previous workers, to discuss the fate of aluminium after it has entered the body.

For this purpose a dog (G) was selected, whose blood content of aluminium was previously found to be too small to be detected by our methods. The dog was then fed for one month on a diet similar to the one previously described, but with the substitution of freshly precipitated, moist aluminium phosphate for the bone ash of the normal diet. This diet is somewhat deficient in calcium.

The fact that aluminium, even if administered in a more soluble form, would be changed to phosphate in the upper small intestine, led to the choice of the phosphate as the form in which to feed the aluminium. In addition, any chances for acidosis, or of a toxic effect of the anion attendant on feeding water soluble aluminium was thus eliminated. The body weight of the dog at the beginning of the experiment was 14.0 kilos; at the end 12.3 kilos. A dose of 10. grams of aluminium phosphate per day was found to be the maximum which could be administered without causing diarrhea. The health of the animal was apparently not particularly injured by the experiment, although a decrease in body weight and a loss of appetite were apparent, particularly toward the last.

The dog was bled to death and perfused in the manner already explained. Analyses of the tissues were made for aluminium as before. The results are recorded in Table V. The presence of aluminium in these precipitates was shown in each case with Goppsroeder's test. All these precipitates of AlPO_4 were white, in-

fusible, and, when tested for calcium by being moistened with hydrochloric acid, and introduced on the end of a platinum wire into the non-luminous flame of a bunsen burner, imparted no color to the flame.

TABLE V.

Amount of aluminium occurring in the tissues of dog G, fed on freshly precipitated AlPO_4 . The different columns and their numbering have the same significance as in Table IV.

I.	II.	III.	IV.	V.	VI	VII.
G. Blood before experiment		330. g.	Neumann	0.0 mg.	0.00 mg.	0.00 mg.
Blood, after experiment (Plasma)		625.	"	1.4	0.59	0.09
Blood, after experiment (Corpuscles)		150.	"	1.5	0.63	0.42
Bile + Gall						
Bladder		35.	"	1.5	0.63	1.80
Spleen		30.	"	0.0	0.00	0.00
Kidneys (2)		85.	"	3.2	1.34	1.58
Muscle (Leg)		57.	"	0.2	0.08	0.15
Bone (Femur)						
Freed from marrow		10.	Ignition	0.6	0.25	2.5
Bone marrow (Femur)		8.	"	1.1	0.46	5.8
Claws		4.	"	0.0	0.00	0.0
Ferratin, from liver		0.348	"	0.5	0.21	60.
Remainder of liver		240.	Neumann	0.4	0.17	0.07

As was described in the footnote to Table IV., blanks were run and the appropriate corrections made.

An element in the animal body can always be detected when present, by the use of appropriate analytical means. No matter how involved the chemical changes in which it may take part, it is always possible to find the element with proper methods. After absorption such an element would necessarily occur in the blood. The blood analyses would therefore furnish evidence for or against the passage of aluminium into the body. Since this metal is foreign to the body there will be a tendency toward elimination, and

both the excretory organs and their excretions may be expected to contain the metal in comparatively large amount. For this reason the kidneys, the liver, and the contents of the gall bladder were also selected for analysis. If, however, elimination is not so rapid as absorption, storage must occur, and the liver, from its tendency to store metals would be the most probable place for this accumulation. On the other hand there is always the possibility that some tissue may possess a particular retentive power for the metal in question, and to investigate this, samples were taken of muscle (from the leg), of a typical keratin (the claws), of bone (the femur), and of one of the glands of internal secretion (the spleen).

Our results show most of the aluminium to be speedily eliminated, through the kidneys and by the liver. Analyses of the urine of human beings fed with aluminized bread, show considerable aluminium and are in agreement with this finding¹. There is, however, a storage of aluminium, at least temporary, which is not confined to any one particular tissue. The distribution is not uniform, however, and seems to be roughly parallel to that of the iron.

Iron is the only trivalent metal normally found in the body, and as far as we know, it occurs principally combined with proteins. Would it be possible for the closely related trivalent aluminium to replace part of the iron in such combinations, in much the same way, perhaps, as this occurs in the mineral world? To prove this absolutely would be difficult indeed, for tissues are like composite rocks rather than single minerals with definite empirical formulae, and any comparison involving the total amount of iron present would be utterly worthless.

Evidence pointing strongly to such replacement, however, is not lacking. If the blood be mixed, at the time of collection, with sodium oxalate dissolved in physiological salt solution, clotting is prevented, and the corpuscles may be separated from the plasma by centrifuging. The concentration of aluminium in the corpuscles was found to be much higher than in the iron-free plasma. Now in adult animals not suffering from anaemia, the red corpuscles are synthesized in the marrow of the long bones. Accordingly, the femur was taken from the dog's body, split, and the marrow, including as much of the spongy portion of the bones as possible, was removed with a steel instrument. Both the bone and the marrow contained aluminium but the latter did so in very large

¹Paper in process of publication.

quantities. The presence of the metal in the red corpuscles, therefore, dates from the formation of the cell, and can be regarded as an original constituent rather than as a subsequently acquired "impurity."

Furthermore, the erythrocytes are broken down in the liver and the iron formerly contained in the haemoglobin is stored there, in a great part as ferruginous proteins, the so-called "ferratins."

Ferratin was accordingly prepared from the liver. The method used was that of Wohlgemuth², which is essentially the same as the original procedure of Schmiedeberg.³

The fresh, finely divided liver was covered with water, and the temperature gradually increased to boiling, when the aqueous extract was removed. Subsequent extractions with boiling water were then made. The protein was precipitated from the extract with dilute acetic acid and purified by dissolving in ammonia and reprecipitating with acid. The preparation was finally washed with absolute alcohol, with absolute ether, and dried. It was of a light yellow color, and contained on analysis, 4.3% Fe_2O_3 , resembling the ferratin prepared for comparison from the liver of normal dog F. (Section III.), which, however, contained only 2.4% Fe_2O_3 . The aluminium content of the former preparation, (from dog G.), while not very accurately determined on account of the small sample of material available, was at any rate very large.

Aluminium can therefore be seen to follow the iron from the synthesis of haemoglobin in the bone marrow to its disintegration in the liver and the storage of the iron as ferratin. That there is an "aluminium circulation," comparable to the iron circulation well known to physiologists, is then certain. Were this association of aluminium with the iron merely a physical one, it is not reasonable to believe that it would persist through such profound chemical changes. The replacement of protein-combined iron by aluminium, and the formation therefore of "aluminium-proteins," such as we undoubtedly obtained in the case of our specimen of ferratin, seems to be the only logical explanation of the facts.

The presence of aluminium in the bone itself is interesting as recalling the observation of Papillon, already mentioned. The quantity found by him is enormous in comparison, however. It will be remembered, that Papillon's experiments were made on a young animal. The diet used by us would probably not affect

²Wohlgemuth: Zeit. Physiol. Chem xxxvii, 475, (1903).

³Schmiedeberg: Archiv fur Exp. Path. u. Pharm. xxxiii, 101, (1894).

greatly the composition of the bones of an adult animal, whose skeleton had already fully developed. It was thought advisable therefore, to repeat the work on a puppy, where this lack of bone-building substances would be more marked, owing to the absence of milk from the food, and where the bones were still in the process of rapid growth. This experiment is still in progress (May 12, 1917).

The principle of aiding substitution by creating a deficiency of the material to be replaced in the diet, was evidently not correctly applied by Koenig⁴. Since the aluminium replaces the iron rather than the calcium of the body, a diet deficient in iron, not calcium, is indicated, and experiments on an iron-poor diet would make an interesting chapter to the investigation.

We may conclude then that aluminium is absorbed from the digestive tract of the dog in considerable quantities when fed as aluminium phosphate.

Much of this aluminium is speedily eliminated in the bile and the urine. Some, however, is retained in the body, in all probability replacing the iron of ferruginous proteins, and accompanies this iron in its cycle: Bone Marrow—Red Corpuscles—Ferratin—Bone Marrow. Still another portion remains in the bones, and, although to a much smaller extent than found by Papillon, confirms his results.

VI.

Is Aluminium Absorbed by Dogs from Aluminized Bread?

The experiments described here were undertaken to determine whether or not aluminium is absorbed in dogs fed on a diet containing "aluminized food." It was planned to repeat, in a general way, experiments previously conducted in this laboratory¹, but to use, in the determination of aluminium, the method devised by Schmidt and Hoagland².

The experiments were performed on two dogs (A and B), which were fed aluminized food for a period of about three months. The dogs had been previously maintained on a definite diet of hashed lean beef, cracker meal, infusorial earth and water. During the experiment "baking-powder biscuits," made from alum baking-powder, according to Kahn³, were substituted for cracker

¹J. Konig: loc. cit.

²Kahn: loc. cit.

³Gies: Biochem Bull. v, (1916).

⁴Kahn: loc. cit. The ingredients used were the same as used in preparing the bread used in Section II.

meal, and in such quantity as to give each dog, daily, about 20. mg. of aluminium as Al_2O_3 , per kilo of body weight.

Representative data, showing the general condition of the dogs at weekly intervals throughout the experiments, are recorded in Table VI.

TABLE VI.

Data showing the weight and condition of the urine of dogs A and B during the experiments.⁴

Week of the ex- periment	Weight of dog in kilos	DOG A		Weight of dog in kilos	DOG B	
		Urine			Urine	
		48 hour sample			48 hour sample	
		Vol. (cc).	Sp. Gr.		Vol. (cc).	Sp. Gr.
1.	13.9	...		9.9	...	
2.	13.8	520.	1.043	9.3	425.	1.023
3.	13.4	550.	1.044	8.7	240.	1.030
4.	13.3	565.	1.043	9.0	310.	1.043
5. ⁵	13.0	450.	1.052	9.1	450.	1.032
6.	13.0	500.	1.039	8.9	390.	1.035
7.	13.1	460.	1.036	8.5	450. ⁸	1.037
8.	13.0	590. ⁶		8.4	...	
9.	12.9	...		8.9	400.	1.033
10.	13.8	475.	1.033	8.8	580.	1.027
11.	14.0	650.	1.032	8.8	375.	1.040
12.	14.1	725.	1.030	..	...	

At the end of the feeding period, each dog was bled to death from a femoral artery and the blood still remaining in the tissues was washed out by a perfusion with physiological salt solution directed into a femoral vein. The blood, also the "blood washings," were analyzed separately. Besides the blood, the liver, contents of the urinary bladder, and contents of the gall bladder, of each animal, were subjected to analysis for aluminium.

In preparation for analysis the material was placed in a Kjeldahl flask and decomposed by the Neumann process, with nitric and sulphuric acids, and was then treated as outlined in III.

⁴The initial diet consisted (per kilo of body weight) of hashed lean beef, 15. g., "baking-powder biscuits," 15. g., infusorial earth, 1. g., water 20. cc.

⁵Lard added, 1. g. per kilo. (Dog A.)

⁶Water increased to 30. cc. per kilo per day.

The analytical results, obtained by the Schmidt-Hoagland method, are summarized in Table VII.

TABLE VII.

Data pertaining to the amount of aluminium absorbed, in dogs fed on a diet containing aluminized biscuit.

DOG A.				
Part	Weight of material used.	Weight ⁷ of AlPO_4	Weight of Al_2O_3 Total.	Per 100. g. material
Blood	960. g.	1.3 mg.	0.5 mg.	0.06 mg.
"Blood-washings" 740. ⁸		0.5	0.1	...
Urine (in bladder) 64.		0.5	0.2	0.35
Bile 9. ⁹		0.2	0.1	0.93
Liver 320.		0.8	0.3	0.10
DOG B.				
Blood	420. g.	3.0 mg.	1.3 mg.	0.30 mg.
"Blood-washings" 850.		0.8	0.3	...
Urine (in bladder) 14.		0.6	0.3	1.65
Bile 3.		0.9	0.4	12.55
Liver 240.		0.8	0.3	0.14

The phosphates of aluminium thus obtained were white or at most faintly tinged with yellow. They were infusible at white heat, except occasionally when a colorless or slightly greenish glass was formed, suggesting the presence of traces of iron. These precipitates are comparatively insoluble in ordinary reagents, but hot concentrated phosphoric or sulfuric acids dissolve them readily.

Three control determinations, by the same method, were made in the glassware and with the supplies of reagents used for the estimations referred to in Table VII. The following weights of aluminium phosphate (mg.) were obtained: 0.2, 0.0, and 0.1; average, 0.1.

⁷The blank corrections, referred to above, have been applied to these weights.

⁸The blood washings were mainly salt solution, of course. Estimated on the assumption that 1-12 of the body weight was blood and that all was flushed out, the amount of blood in the washings was 217. cc. for dog A and 310. cc. for dog B.

⁹Bile and gall bladder.

The salt solution used in obtaining the "blood washings," and the distilled water used throughout the work were found to be free from aluminium.

After recording the weights of the precipitates of aluminium phosphate (Table VII.) each precipitate was subjected to qualitative analysis for aluminium. For this purpose we used a modification of the Goppelsroeder test.¹⁰

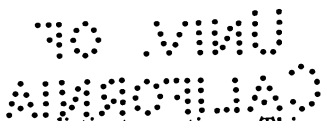
Goppelsroeder's test depends upon the development of an intense green fluorescence when an alcoholic solution containing aluminium is mixed with "morin" dissolved in alcohol. Morin is prepared from an aqueous extract of fustic wood. The extract is evaporated to small volume and impure morin separates out. This is filtered off, and purified by fractional crystallization from alcohol. Iron does not affect the reaction, if the preparation of morin is pure, but the test is inhibited by the presence of some free acids, although acetic acid in fairly large proportion or traces of hydrochloric acid seem, however, to be without effect.

In order to adapt this test to the precipitates of AlPO_4 and, at the same time, to prevent access of the small amounts of aluminium apt to be introduced during the manipulation, the following procedure was adopted: A few drops of hydrochloric acid solution were evaporated nearly to dryness on a watch glass over a small water bath; 1-2 cc. of alcohol were added and allowed to evaporate nearly to dryness thus removing most of the acid. About 1-2 cc. of alcohol were again added and the solution removed with a capillary pipet to a small test tube. To this a drop of 1% alcoholic morin solution was added, and the mixture allowed to stand for some minutes. When this reaction was negative, the reagents and glassware failed to yield soluble aluminium. In the latter event, a small amount of the phosphate precipitate was then placed on the watch glass and the whole process repeated in the tested pipet and test tube. When aluminium was present in the alcoholic solution, a strong green fluorescence developed in a few minutes. It was best observed by reflected light, from a source rich in blue or violet rays.

All the precipitates obtained were tested according to this technic and invariably contained aluminium.

The ignited phosphate was never completely decomposed by this procedure, but sufficient AlCl_3 was obtained in each test to give a

¹⁰Goppelsroeder: *Zeit. Anal. Ch.* vii, 195, (1868). See also Balls: *Biochem. Bull.* v, (1916).



distinct reaction. This test when conducted in this way, detects 0.01 mg. of Al_2O_3 in such precipitates.

Unless all glassware used in this test is previously boiled in hydrochloric acid solution and washed with distilled water, the controls will invariably show a content of aluminium, and the small precipitates obtained in the "blank determinations" may do so likewise. In these cases, the fluorescence will be very much less intense. Weakly positive tests, therefore, have been uniformly regarded as negative.

In reviewing the results, recorded in Table VII., and confirmed qualitatively with Goppelsroeder's test, it is interesting to note that although dog B. was the smaller animal, the quantities of aluminium found in his tissues were larger than in the case of dog A.. Both dogs were in good health but A was undoubtedly the more vigorous animal.

Conclusions: The blood, liver, bile, and urine from each of two dogs fed upon a diet including biscuits baked with alum baking powder, contained aluminium in every instance.

These findings corroborate those of Steel and Kahn. They prove that aluminium is absorbed in dogs from a diet containing aluminized bread.

Summary

We may briefly summarize our findings as follows:

1. The method of Schmidt and Hoagland for the determination of aluminium, in which the aluminium is precipitated and weighed as AlPO_4 , gives low results. The addition of ammonium phosphate to the water used for washing is recommended.
2. From bread baked with aluminium baking powder practically all the aluminium is extracted by "artificial gastric juice." After gastric digestion of such bread, some aluminium still remain dissolved when the duodenal conditions affecting the digested mixture are simulated in vitro.
3. In normal dogs, aluminium exists, if at all, in amounts too small to be demonstrable by the best available methods.
4. Aluminium is absorbed by dogs from food containing aluminium phosphate, and from bread baked with alum baking powder. Much of this aluminium is speedily eliminated, but some is retained, replacing part of the iron occurring normally in the tissues.

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Vita

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Previous Publications:

The Electrolytic Separation of Zinc, Copper and Iron from Arsenic. Jour. Ind. and Eng. Chem. vii p. 26 (1915) (with C. C. McDonnell).

The accuracy of the Schmidt-Hoagland method for the determination of Aluminium. Biochem. Bull V, p. 195 (1916).

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